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L1 6786 SEA FILE=REGISTRY (ASPERGILLUM/BI OR ASPERGILLUS/BI OR ASPERGILUS/BI)
L2 22689 SEA FILE=HCAPLUS L1
L3 51784 SEA FILE=HCAPLUS L2 OR FILAMENT?(W) FUNG? OR ASPERGILL?
L4 7534 SEA FILE=HCAPLUS L3 AND (?NUCLE? OR RNA OR DNA OR DEXOYRIB? OR RIBONUCL?)
L5 506 SEA FILE=HCAPLUS L4 AND LIBRAR?
L6 155 SEA FILE=HCAPLUS L5 AND (MARKER? OR VECTOR?)
L7 28712 SEA FILE=HCAPLUS LIBRAR? (L) (?NUCLE? OR RNA OR DNA OR DEXOYRIB? OR RIBONUCL?)
L10 1 SEA FILE=REGISTRY "ORNITHINE CARBAMOYLTRANSFERASE"/CN
L11 7621 SEA FILE=REGISTRY SYNTHASE/BI
L12 6376 SEA FILE=REGISTRY REDUCTASE/BI
L13 1886 SEA FILE=REGISTRY CARBOXYLASE/BI
L14 23012 SEA FILE=REGISTRY TRANSFERASE/BI
L15 28 SEA FILE=REGISTRY ACETAMIDASE/BI
L16 878 SEA FILE=REGISTRY PERMEASE
L17 467578 SEA FILE=HCAPLUS L10 OR ?TRANSFERASE? OR SYNTHASE? OR REDUCTASE ? OR CARBOXYLAS? OR ACETAMIDAS? OR PERMEAS? OR L11 OR L12 OR L13 OR L14 OR L15 OR L16
L21 16512 SEA FILE=HCAPLUS (SELECT? OR SCREEN? OR CONSTRUCT?) AND L7
L22 248 SEA FILE=HCAPLUS L21 AND L3
L23 81 SEA FILE=HCAPLUS L22 AND L17
L24 384 SEA FILE=HCAPLUS L3(L) LIBRAR?
L25 60 SEA FILE=HCAPLUS L24 AND L23

L26

30 SEA FILE=HCAPLUS L6 AND L25

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L26 ANSWER 1 OF 30 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:291226 HCAPLUS
DOCUMENT NUMBER: 132:319501
TITLE: Methods of **constructing** and
screening a DNA library of
interest in filamentous fungal
cells
INVENTOR(S): Vind, Jesper
PATENT ASSIGNEE(S): Novo Nordisk A/s, Den.
SOURCE: PCT Int. Appl., 81 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000024883	A1	20000504	WO 1999-DK552	19991013
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 9961885	A1	20000515	AU 1999-61885	19991013
PRIORITY APPLN. INFO.:			DK 1998-1375	19981026
			DK 1999-718	19990525
			WO 1999-DK552	19991013

AB The invention provides a method of **constructing** and **screening a library of polynucleotide** sequences of interest in **filamentous fungal cells** by use of an episomal replicating AMAl-based plasmid **vector**, thus achieving a high frequency of transformation and a stable and std. uniformly high level of gene expression.

IT 267218-07-5 267218-08-6
RL: BPR (Biological process); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(**nucleotide sequence; methods of constructing and screening a DNA library of interest in filamentous fungal cells**)
IT 9001-92-7P, Proteolytic enzyme 9002-10-2P, Polyphenoloxidase 9003-99-0P, Peroxidase 9030-09-5P, Cyclodextrin **glycosyltransferase** 9032-08-0P, Glucoamylase 9047-61-4P, **Transferase** 9055-15-6P, Oxidoreductase 80146-85-6P, Transglutaminase
RL: ARU (Analytical role, unclassified); BPN (Biosynthetic preparation);

M. Smith 308-3278

ANST (Analytical study); BIOL (Biological study); PREP (Preparation)
(screening for; methods of constructing and
screening a DNA library of interest in
filamentous fungal cells)

IT 9000-90-2P, Taka amylase

RL: BPN (Biosynthetic preparation); BPR (Biological process); BIOL
(Biological study); PREP (Preparation); PROC (Process)
(vector comprising gene promoter of; methods of
constructing and screening a DNA
library of interest in filamentous fungal
cells)

REFERENCE COUNT:

4

REFERENCE(S):

- (1) Aleksenko, A; Fungal Genetics and Biology 1997, V21, P373 HCAPLUS
- (2) Aleksenko, A; Mol Gen Genet 1996, V253, P242 HCAPLUS
- (3) Alexei, A; Molecular Microbiology 1996, V20(2), P427
- (4) Gems, D; Curr Genet 1993, V24, P520 HCAPLUS

L26 ANSWER 2 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:259069 HCAPLUS

DOCUMENT NUMBER: 133:70531

TITLE:

Aristolochene **Synthase**: Purification,
Molecular Cloning, High-Level Expression in
Escherichia coli, and Characterization of the
Aspergillus terreus Cyclase

AUTHOR(S):

Cane, David E.; Kang, Ilgu

CORPORATE SOURCE:

Department of Chemistry, Box H, Brown University,
Providence, RI, 02912-9108, USA

SOURCE:

Arch. Biochem. Biophys. (2000), 376(2), 354-364
CODEN: ABBIA4; ISSN: 0003-9861

PUBLISHER:

Academic Press

DOCUMENT TYPE:

Journal

LANGUAGE:

English

AB Aristolochene **synthase** catalyzes the cyclization of farnesyl diphosphate to (+)-aristolochene. The **Aspergillus terreus** enzyme has been purified 75-fold to homogeneity in six steps. Based on the sequence of 3 internal peptides obtained by Lys-C digestion of the native protein, a set of degenerate PCR primers was used to amplify a 550-bp segment of cDNA corresponding to a portion of the aristolochene **synthase** transcript. A second round of PCR using specific primers was used to prep. a 32P-labeled 180-bp segment, which was used to screen an A. terreus cDNA library prepd. using .lambda.ZapII, resulting in the identification and sequencing of the A. terreus aristolochene **synthase** cDNA. Aristolochene **synthase** was encoded by an open reading frame (ORF) of 960 bp, corresponding to a protein of 320 amino acids with a predicted MD of 36,480. Comparison of the A. terreus ORF with the sequence of the previously described aristolochene **synthase** from Penicillium roqueforti revealed a 66% of identity at the nucleic acid level and a 70% identity at the deduced amino acid level between the aristolochene **synthases** from the two different fungal sources. PCR was used to insert the A. terreus aristolochene **synthase** gene into the T7lac expression vector pET11a. Cloning of the resultant construct into Escherichia coli XL1-Blue and

subcloning into the expression host *E. coli* BL21(DE3)/pLySS gave, after induction with IPTG, sol. aristolochene **synthase** as 5-10% of total protein. The recombinant aristolochene **synthase**, which was purified 13-fold to homogeneity, appeared to be identical in all respects with the native *A. terreus* enzyme, displaying essentially the same steady-state kinetic parameters, with a K_m of 15 nM and k_{cat} 0.015 s⁻¹. Using PCR to amplify the aristolochene **synthase** gene (Arl) from *A. terreus* genomic DNA revealed the presence of 2 introns, identical in relative location but different in both sequence and length compared to the corresponding Aril gene of *P. roqueforti*. (c) 2000 Academic Press.

IT 278814-37-2

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(amino acid sequence; purifn., mol. cloning, high-level expression in *E. coli*, and characterization of *Aspergillus terreus* aristolochene **synthase**)

IT 249497-95-8, GenBank AF198359 249497-96-9, GenBank AF198360

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(**nucleotide** sequence; purifn., mol. cloning, high-level expression in *E. coli*, and characterization of *Aspergillus terreus* aristolochene **synthase**)

IT 94185-89-4P, Aristolochene Synthase

RL: BAC (Biological activity or effector, except adverse); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)

(purifn., mol. cloning, high-level expression in *E. coli*, and characterization of *Aspergillus terreus* aristolochene **synthase**)

REFERENCE COUNT:

37

REFERENCE(S):

- (3) Bohlmann, J; Proc Natl Acad Sci USA 1998, V95, P6756 HCAPLUS
 - (4) Bradford, M; Anal Biochem 1976, V72, P248 HCAPLUS
 - (5) Cane, D; Arch Biochem Biophys 1993, V304, P415 HCAPLUS
 - (6) Cane, D; Arch Biochem Biophys 1993, V300, P416 HCAPLUS
 - (7) Cane, D; Biochemistry 1994, V33, P5846 HCAPLUS
- ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 3 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:241502 HCAPLUS

DOCUMENT NUMBER: 132:275163

TITLE: A fungal transcriptional activator and the gene encoding it and their use in expression of foreign genes in fungal hosts

INVENTOR(S): Hjort, Carsten; Van Den Hondel, Cees A. M. J. J.; Punt, Peter J.; Schuren, Frank H. J.

PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.

SOURCE: PCT Int. Appl., 86 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000020596	A1	20000413	WO 1999-DK524	19991005
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9958509	A1	20000426	AU 1999-58509	19991005
PRIORITY APPLN. INFO.:			DK 1998-1258	19981005
			WO 1999-DK524	19991005

AB The prtT gene required for expression of the pepA gene of *Aspergillus niger* is cloned and characterized for use in expression systems in yeasts and filamentous fungi. The gene was cloned by expression in *Aspergillus niger*. A prtT-deficient mutant was constructed with an amdS gene under control of the pepA promoter. This was transformed with a cosmid library in an autonomously replicating vector carrying a pyrG selective marker. Transformants were selected for acetamide utilization and the pyrG marker and plasmid DNA recovered and subcloned to identify a 2.5 kb PstI fragment that carried the gene. Sequencing of the gene and anal. of the translation product indicated that the protein is a member of the GAL4 family of transcription factors.

IT 263740-31-4

RL: BPR (Biological process); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process); USES (Uses) (amino acid sequence; fungal transcriptional activator and gene encoding it and their use in expression of foreign genes in fungal hosts)

IT 9025-49-4P, Aspergillopepsin A 9074-07-1P

RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation) (gene for, expression in filamentous fungi of; fungal transcriptional activator and gene encoding it and their use in expression of foreign genes in fungal hosts)

IT 9001-92-7P, Proteinase 9002-10-2P, Polyphenol oxidase

9003-99-0P, Peroxidase 9047-61-4P, Transferase

9055-15-6P, Oxidoreductase 80146-85-6P, Transglutaminase

RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)

(manuf. in fungal hosts of; fungal transcriptional activator and gene encoding it and their use in expression of foreign genes in fungal hosts)

IT 263740-30-3

RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(nucleotide sequence; fungal transcriptional activator and gene encoding it and their use in expression of foreign genes in fungal hosts)

REFERENCE COUNT: 6
 REFERENCE(S): (1) Bibbins, M; SWISS-PROT database 1997
 (2) Burger, G; Mol Cell Biol 1991, V11, P5746 HCAPLUS
 (3) Burger, G; SWISS-PROT database
 (4) Entian, K; SWISS-PROT database 1994
 (6) Purnelle, B; Yeast 1994, V10, P1235 HCAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 4 OF 30 HCAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 2000:85038 HCAPLUS
 DOCUMENT NUMBER: 132:133197
 TITLE: Novel methods for in vivo identification of enzyme inhibitors from random peptide-chymotrypsin inhibitor 2A (CI-2A) fusion library and their use in drug screening
 INVENTOR(S): Halkier, Torben; Jespersen, Lene; Jensen, Allan
 PATENT ASSIGNEE(S): M & E Biotech A/S, Den.
 SOURCE: PCT Int. Appl., 136 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000005406	A1	20000203	WO 1999-DK408	19990716
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9958985	A1	20000214	AU 1999-48985	19990716
PRIORITY APPLN. INFO.:			DK 1998-956	19980720
			US 1998-94868	19980729
			WO 1999-DK408	19990716

AB Novel methods (so called CellScreen.RTM. technol.) for in vivo identification enzyme inhibitors from random peptide-chymotrypsin inhibitor 2A (CI-2A) fusion library and their use in drug screening are described. Barley CI-2A from the potato inhibitor I family of protease inhibitors is used as the scaffold to display random peptide sequences in vivo since it can be stably and sufficiently expressed in the nucleus or ER of cultured cells, or displayed on the phage particles and remains biol. active. Random peptide library is constructed by inserting the random synthetic oligonucleotides or PCR fragments inside the CI-2A loop coding region in the retroviral expression vector and expressed intracellularly. The signal peptide sequence for various intracellular compartments or peptide tag can be fused at the N-terminus of the peptide-CI-2A library for the localization or purifn. purpose. The enzyme inhibitors or their relative RNA can be isolated from the phenotypically altered cells and used for further screening of their interaction partners which has therapeutic potentials.

IT 50812-37-8, **Glutathione-S-transferase**
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
 (Uses)
 (epitope tag from; novel methods for in vivo identification of enzyme
 inhibitors from random peptide-chymotrypsin inhibitor 2A (CI-2A) fusion
library and their use in drug **screening**)

IT 9000-90-2P, .alpha.-Amylase 9014-24-8P, RNA
 polymerase 9032-75-1P, Polygalacturonase 9073-60-3P,
 .beta.-Lactamase 56626-18-7P, **Fucosyltransferase**
 RL: BPN (Biosynthetic preparation); PUR (Purification or recovery); THU
 (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES
 (Uses)
 (inhibitor of; novel methods for in vivo identification of enzyme
 inhibitors from random peptide-chymotrypsin inhibitor 2A (CI-2A) fusion
library and their use in drug **screening**)

REFERENCE COUNT: 6
 REFERENCE(S):
 (1) Blind, M; PROC NATL ACAD SCI USA 1999, V96 HCAPLUS
 (2) Ferber, M; J MOL BIOL 1998, V279, P565 HCAPLUS
 (3) Jack, B; WO 9832880 A 1998 HCAPLUS
 (4) Klug, S; PROC NATL ACAD SCI USA 1997, V94, P6676
 HCAPLUS
 (5) Mouritsen; WO 9638553 A 1996 HCAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 5 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:749748 HCAPLUS

DOCUMENT NUMBER: 132:103549

TITLE: Cloning and sequencing of the chromosomal **DNA**
 and cDNA encoding the mitochondrial citrate
synthase of **Aspergillus niger**

WU-2223L

AUTHOR(S): Kirimura, Kohtaro; Yoda, Masashi; Ko, Ikuyo; Oshida,
 Yuichi; Miyake, Kouichiro; Usami, Shoji

CORPORATE SOURCE: Department of Applied Chemistry, School of Science and
 Engineering, Waseda University, Tokyo, 169-8555, Japan

SOURCE: J. Biosci. Bioeng. (1999), 88(3), 237-243

CODEN: JBBIF6; ISSN: 1389-1723

PUBLISHER: Society for Bioscience and Bioengineering, Japan

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The cDNA and chromosomal **DNA** encoding the citrate
synthase (EC 4.1.3.7) gene (cit1) of **Aspergillus niger**
 WU-2223L, a citric acid-producing strain, were cloned. Synthetic
oligonucleotide primers were designed according to the amino acid
 sequences of already known eukaryotic citrate **synthases** and the
 codon bias of A. niger genes. The 920-bp **DNA** fragment was
 amplified by PCR with these primers using chromosomal **DNA** of
 WU-2223L as a template, and was employed to **screen** a cDNA
library of A. niger. One full-length cDNA clone was isolated and
 sequenced, within which an ORF of 1425 bp encoding a protein of 475 amino
 acids with a mol. wt. of 52,153 Da was found. Its N-terminal region
 contains a typical mitochondrial-targeting motif. The predicted amino
 acid sequence was 82, 68, and 65% homologous with the mitochondrial
 citrate **synthases** of Neurospora crassa, Saccharomyces
 cerevisiae, and pig, resp., but it showed lower homol. to bacterial
 citrate **synthases**. The full-length cDNA clone was used to

screen a chromosomal library of *A. niger* WU-2223L, and a 7.5-kb SalI fragment contg. the corresponding chromosomal gene was isolated. Comparison of the chromosomal and cDNA sequences revealed that the *cit1* gene is interrupted by six introns. In the chromosomal DNA, upstream of the coding region, a CT-rich region, but not the TATAAA or CAAT motifs, was found. *Escherichia coli* MOB150, a citrate synthase-deficient mutant showing a glutamate-requiring phenotype, was transformed with the plasmid pKAC-35S, which is the expression vector pKK223-3 contg. the cDNA fragment encoding a putative mature protein of *A. niger* citrate synthase. The transformant harboring pKAC-35S showed citrate synthase activity and a glutamate-nonrequiring phenotype.

IT 255853-95-3

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; cloning and sequencing of the chromosomal DNA and cDNA encoding the mitochondrial citrate synthase of *Aspergillus niger* WU-2223L)

IT 9027-96-7, Citrate synthase

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(cloning and sequencing of the chromosomal DNA and cDNA encoding the mitochondrial citrate synthase of *Aspergillus niger* WU-2223L)

IT 167229-74-5, GenBank D63376

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; cloning and sequencing of the chromosomal DNA and cDNA encoding the mitochondrial citrate synthase of *Aspergillus niger* WU-2223L)

REFERENCE COUNT: 27

REFERENCE(S):

- (1) Bhayana, V; Biochemistry 1984, V23, P2900 HCAPLUS
 - (2) Chirgwin, J; Biochemistry 1979, V18, P5294 HCAPLUS
 - (3) Evans, C; Biochemistry 1988, V27, P4680 HCAPLUS
 - (4) Ferea, T; Mol Gen Genet 1994, V242, P105 HCAPLUS
 - (5) Fukaya, M; J Bacteriol 1990, V172, P2096 HCAPLUS
- ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 6 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:592769 HCAPLUS

DOCUMENT NUMBER: 132:147496

TITLE: Cloning and sequences comparison of promoters from

Aspergillus niger

AUTHOR(S): Luo, Xin-Mei; Schoenherr, R.; Chen, Hong

CORPORATE SOURCE: Max Planck Research Group "MZB", Jena, D-07747, Germany

SOURCE: Yichuan Xuebao (1999), 26(4), 428-436

CODEN: ICHPCG; ISSN: 0379-4172

PUBLISHER: Kexue Chubanshe

DOCUMENT TYPE: Journal

LANGUAGE: Chinese

AB An *Aspergillus niger* genomic library was constructed in a promoter-trap vector, which contains a hygromycin B phosphotransferase-encoding gene (*hph*) and screened for DNA fragments with promoter activity by applying the sib selection procedure. A functional promoter

PX27 was identified. Both DNA strands of this fragment were sequenced and showed no significant homol. to the sequence already in the database. Comparison of the sequences of all known promoters from A. niger revealed a consensus CTTCTC, as a novel motif of the A. niger promoters.

IT 188219-96-7, GenBank U90936
 RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
 (nucleotide sequence; cloning and DNA sequence of promoter PX27 from *Aspergillus niger*)

L26 ANSWER 7 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:90259 HCAPLUS
 DOCUMENT NUMBER: 130:150355
 TITLE: *Aspergillus* porphobilinogen
 synthases and nucleic acids encoding
 the enzyme
 INVENTOR(S): Jones, Aubrey; Cherry, Joel R.
 PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
 SOURCE: U.S., 29 pp.
 CODEN: USXXAM
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	US 5866391	A	19990202	US 1997-871268	19970609
AB	The present invention relates to <i>Aspergillus</i> porphobilinogen synthases and isolated nucleic acid fragments comprising nucleic acid sequences encoding the porphobilinogen synthases as well as nucleic acid constructs, vectors, and recombinant host cells comprising the nucleic acid sequences. Thus, a genomic hemB probe was generated by PCR and used to identify prophobilinogen synthase hemB clones is DNA genomic libraries from <i>Aspergillus oryzae</i> strain A1560 (IFO 4177). The nucleotide sequence of the cloned A. oryzae hemB gene reveals an open reading frame of 1308 nucleotides encoding a 374-amino acid polypeptide with a predicted mol. wt. of 40 kDa. The nucleotide sequence contains one 48-bp putative intron which is flanked by splice site consensus sequences and contains an internal consensus sequence. The invention also relates to methods of producing the porphobilinogen synthases.				
IT	9036-37-7P, Porphobilinogen synthase RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation) (<i>Aspergillus</i> porphobilinogen synthases and nucleic acids encoding the enzyme)				
IT	200890-35-3P RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation) (amino acid sequence; <i>Aspergillus</i> porphobilinogen synthases and nucleic acids encoding the enzyme)				
IT	200890-34-2P RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological				

study); PREP (Preparation)

(**nucleotide** sequence; **Aspergillus** porphobilinogen
synthases and **nucleic** acids encoding the enzyme)

REFERENCE COUNT: 5

REFERENCE(S): (1) Anon; WO 9303185 1993 HCAPLUS
(2) Jaffe, E; Journal of Bioenergetics and
Biomembranes 1995, V27(2) HCAPLUS
(3) Mitchell; Journal of Biological Chemistry 1995,
V270(41), P24054 HCAPLUS
(4) Myers; J Biol Chem 1987, V262(35), P16822 HCAPLUS
(5) Myers; Journal of Biological Chemistry 1987,
V262(35), P16822 HCAPLUS

L26 ANSWER 8 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:549705 HCAPLUS

DOCUMENT NUMBER: 130:974

TITLE: Cloning of the nitrate **reductase** gene of
Stagonospora (Septoria) nodorum and its use as a
selectable marker for targeted
transformation

AUTHOR(S): Cutler, S. B.; Cooley, R. Neil; Caten, Christopher E.

CORPORATE SOURCE: School of Biological Sciences, The University of
Birmingham, Edgbaston, Birmingham, B15 2TT, UK

SOURCE: Curr. Genet. (1998), 34(2), 128-137

CODEN: CUGED5; ISSN: 0172-8083

PUBLISHER: Springer-Verlag

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The nitrate **reductase** gene (NIA1) of the phytopathogenic fungus
Stagonospora (Septoria) nodorum has been cloned from a cosmid
library by homologous hybridization with a PCR-generated probe. A
6.7-kb fragment carrying the NIA1 gene was subcloned and partially
characterized by restriction mapping. Sequencing of the gene indicated a
high degree of homol., both at the **nucleotide** and amino-acid
levels, with nitrate **reductase** genes of other
filamentous fungi. Furthermore, consensus regulatory
signals thought to be involved in the control of nitrogen metab. are
present in the 5' flanking region. The cloned NIA1 gene has been used to
develop a gene-transfer system based on nitrate assimilation. Stable nial
mutants of S. nodorum defective in nitrate **reductase** were
isolated by virtue of their resistance to chlorate. These were
transformed back to nitrate utilisation with the wild-type S. nodorum NIA1
gene. Southern analyses revealed that transformation occurred as a result
of the integration of transforming **DNA** into the fungal genome;
in all cases examd., integration was targeted to the homologous sequence.

IT 215666-63-0

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL
(Biological study)

(amino acid sequence; cloning of the nitrate **reductase** gene
of Stagonospora (Septoria) nodorum and its use as a **selectable**
marker for targeted transformation)

IT 9029-27-0, NADPH-nitrate **reductase**

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL
(Biological study)

(gene NIA1; cloning of the nitrate **reductase** gene of
Stagonospora (Septoria) nodorum and its use as a **selectable**

marker for targeted transformation)

REFERENCE COUNT: 41
 REFERENCE(S): (1) Banks, G; Gene 1993, V131, P69 HCAPLUS
 (3) Campbell, E; Curr Genet 1989, V16, P53 HCAPLUS
 (4) Caten, C; Antifungal agents:discovery and mode of action 1995, P31 HCAPLUS
 (6) Cooley, R; Curr Genet 1988, V13, P383 HCAPLUS
 (7) Cooley, R; J Gen Microbiol 1991, V137, P2085 HCAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 9 OF 30 HCAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 1998:493199 HCAPLUS
 DOCUMENT NUMBER: 129:118769
 TITLE: Identification of genes involved in metabolic pathways and the use of combinatorial DNA libraries to generate novel molecular diversity
 INVENTOR(S): Peterson, Todd C.; Foster, Lyndon M.; Brian, Paul
 PATENT ASSIGNEE(S): Chromaxome Corporation, USA
 SOURCE: U.S., 80 pp. Cont.-in-part of U.S. Ser. No. 639,255.
 CODEN: USXXAM
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 4
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5783431	A	19980721	US 1996-738944	19961024
US 5824485	A	19981020	US 1996-639255	19960424
WO 9817811	A1	19980430	WO 1997-US19958	19971024
W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, HU, ID, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9851632	A1	19980515	AU 1998-51632	19971024
EP 951557	A1	19991027	EP 1997-946473	19971024
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRIORITY APPLN. INFO.:			US 1996-639255	19960424
			US 1995-427244	19950424
			US 1995-427348	19950424
			US 1996-738944	19961024
			WO 1997-US19958	19971024

AB A novel drug discovery system for generating and screening mol. diversity using combinatorial expression libraries of genes from organism manufg. compds. of potential therapeutic use is described. The system provides methods for mixing and cloning genetic materials from a plurality of species of organisms in combinatorial gene expression libraries to generate novel metabolic pathways and classes of compds. The method is applicable to organisms that cannot be easily cultured. The system also provides mobilizable combinatorial gene

expression **libraries** that can be transferred from one species of host organism to another for expression. Also provided are specialized cloning **vectors** for making mobilizable gene expression **libraries**. The system also involves methods for pre-**screening** or identifying for host organisms contg. a **library** that are capable of generating such novel pathways and compds. The method is demonstrated by making **libraries** from Gram-neg. marine bacteria in expression **vectors** for Streptomyces. Colonies identified as hybridizing with probes for genes of polyketide biosynthesis were picked, tested for ability to inhibit bacterial growth and further tested in random combinations. The test identified a no. of combinations that gave rise to antibacteria effects.

L26 ANSWER 10 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:268629 HCAPLUS

DOCUMENT NUMBER: 128:318004

TITLE: Identification of genes involved in metabolic pathways and the use of combinatorial **DNA libraries** to generate novel molecular diversity

INVENTOR(S): Peterson, Todd C.; Foster, Lyndon M.; Brian, Paul

PATENT ASSIGNEE(S): Chromaxome Corp., USA

SOURCE: PCT Int. Appl., 158 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 4

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9817811	A1	19980430	WO 1997-US19958	19971024
W:	AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, HU, ID, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
US 5783431	A	19980721	US 1996-738944	19961024
AU 9851632	A1	19980515	AU 1998-51632	19971024
EP 951557	A1	19991027	EP 1997-946473	19971024
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			

PRIORITY APPLN. INFO.:

US 1996-738944 19961024

US 1996-639255 19960424

WO 1997-US19958 19971024

AB A novel drug discovery system for generating and **screening** mol. diversity using combinatorial expression **libraries** of genes from organism manufg. compds. of potential therapeutic use is described. The system provides methods for mixing and cloning genetic materials from a plurality of species of organisms in combinatorial gene expression **libraries** to generate novel metabolic pathways and classes of compds. The method is applicable to organisms that cannot be easily cultured. The system also provides mobilizable combinatorial gene expression **libraries** that can be transferred from one species of

host organism to another for expression. Also provided are specialized cloning **vectors** for making mobilizable gene expression **libraries**. The system also involves methods for pre-**screening** or identifying for host organisms contg. a **library** that are capable of generating such novel pathways and compds. The method is demonstrated by making **libraries** from Gram-neg. marine bacteria in expression **vectors** for Streptomyces. Colonies identified as hybridizing with probes for genes of polyketide biosynthesis were picked, tested for ability to inhibit bacterial growth and further tested in random combinations. The test identified a no. of combinations that gave rise to antibacteria effects.

L26 ANSWER 11 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:15855 HCAPLUS

DOCUMENT NUMBER: 128:85844

TITLE: **Aspergillus** porphobilinogen
synthases and **nucleic** acids encoding
the enzyme

INVENTOR(S): Jones, Aubrey; Cherry, Joel R.

PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA

SOURCE: PCT Int. Appl., 56 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9747753	A1	19971218	WO 1997-US11014	19970609
W:	AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
AU 9734102	A1	19980107	AU 1997-34102	19970609
EP 907745	A1	19990414	EP 1997-930219	19970609
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI			
CN 1221455	A	19990630	CN 1997-195374	19970609
JP 2000515008	T2	20001114	JP 1998-501900	19970609
PRIORITY APPLN. INFO.:			US 1996-19529	19960610
			WO 1997-US11014	19970609

AB The present invention relates to **Aspergillus** porphobilinogen **synthases** and isolated **nucleic** acid fragments comprising **nucleic** acid sequences encoding the porphobilinogen **synthases** as well as **nucleic** acid **constructs**, **vectors**, and recombinant host cells comprising the **nucleic** acid sequences. Thus, a genomic hemB probe was generated by PCR and used to identify prophobilinogen **synthase** hemB clones is **DNA** genomic **libraries** from **Aspergillus** oryzae strain A1560 (IFO 4177). The **nucleotide** sequence of the cloned A. oryzae hemB gene reveals an open reading frame of 1308 **nucleotides** encoding a 374-amino acid polypeptide with a predicted mol. wt. of 40 kDa. The **nucleotide** sequence contains one 48-bp putative intron which is flanked by splice site consensus sequences and contains an internal

consensus sequence. The invention also relates to methods of producing the porphobilinogen **synthases**.

IT 9036-37-7P, Porphobilinogen **synthase**

RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)

(**Aspergillus** porphobilinogen **synthases** and nucleic acids encoding the enzyme)

IT 200890-35-3P

RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)

(amino acid sequence; **Aspergillus** porphobilinogen **synthases** and nucleic acids encoding the enzyme)

IT 200890-34-2P

RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)

(nucleotide sequence; **Aspergillus** porphobilinogen **synthases** and nucleic acids encoding the enzyme)

L26 ANSWER 12 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:543563 HCAPLUS

DOCUMENT NUMBER: 127:131959

TITLE: A method for in vivo production of a gene

library using an error-prone DNA polymerase and a **selectively** inactivatable, host cell chromosome-replicating DNA polymerase

INVENTOR(S): Borchert, Torben Vedel; Ehrlich, Stanislas Dusko

PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.; Borchert, Torben Vedel; Ehrlich, Stanislas Dusko

SOURCE: PCT Int. Appl., 39 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9725410	A1	19970717	WO 1997-DK14	19970110
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
AU 9713678	A1	19970801	AU 1997-13678	19970110
EP 873398	A1	19981028	EP 1997-900206	19970110
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI			
CN 1210557	A	19990310	CN 1997-191974	19970110
JP 2000502568	T2	20000307	JP 1997-524774	19970110
US 6165718	A	20001226	US 1998-112410	19980708
PRIORITY APPLN. INFO.:			DK 1996-18	19960110
			WO 1997-DK14	19970110

AB A method for in vivo prodn. of a gene **library** in cells wherein

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an error-prone polymerase is used in each ancestral cell to replicate all or a part of a genetic element independently of the host chromosomal replication machinery is disclosed. The genetic element comprises (i) an origin of replication from which replication is initiated, (ii) optionally a genetic **marker**, e.g. a gene conferring resistance towards an antibiotic, (iii) a gene encoding the polypeptide of interest. Also methods for the generation of a **DNA** sequence encoding a desired variant of a polypeptide of interest, and for the detn. of such a **DNA** sequence are described. Thus, *Escherichia coli* contg. a *ts polC* gene mutant, a *polA* gene mutation causing increased error, and a *mutL* mutation causing **DNA** repair deficiency was transformed with pBR322 contg. a frameshift mutation or a stop codon in the *tet* gene. After growth at 37.degree. the culture was shifted to 42.degree. to inhibit **DNA** polymerase III and encourage error-prone **DNA** replication with **DNA** polymerase I. Appearance of tetracycline-resistant colonies indicates presence of a repaired *tet* gene, reflecting a specific mutagenesis event.

IT 9068-38-6, Reverse transcriptase

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(HIV; method for in vivo prodn. of gene **library** using error-prone **DNA** polymerase and **selectively** inactivatable, host cell chromosome-replicating **DNA** polymerase)

IT 9001-92-7P, Protease 9003-99-0P, Peroxidase

9031-48-5P, Glucosyltransferase 9032-75-1P,

Polygalacturonase 9037-80-3P, Reductase

9047-61-4P, Transferase 9054-54-0P,

Acyltransferase 9055-15-6P, Oxidoreductase

RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)

(gene for; method for in vivo prodn. of gene **library** using error-prone **DNA** polymerase and **selectively** inactivatable, host cell chromosome-replicating **DNA** polymerase)

IT 37213-50-6, **DNA** polymerase II

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(method for in vivo prodn. of gene **library** using error-prone **DNA** polymerase and **selectively** inactivatable, host cell chromosome-replicating **DNA** polymerase)

IT 37217-33-7, **DNA** polymerase III

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(mutant *E. coli*; method for in vivo prodn. of gene **library** using error-prone **DNA** polymerase and **selectively** inactivatable, host cell chromosome-replicating **DNA** polymerase)

IT 9012-90-2, **DNA** polymerase

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(of *E. coli*, *B. subtilis*, or phage; method for in vivo prodn. of gene **library** using error-prone **DNA** polymerase and **selectively** inactivatable, host cell chromosome-replicating **DNA** polymerase)

L26 ANSWER 13 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:257466 HCAPLUS

DOCUMENT NUMBER: 126:235236

TITLE: Novel lipolytic enzyme muteins designed for one-wash detergent compositions for the removal of fatty materials

INVENTOR(S): Okkels, Jens Sigurd; Svendsen, Allan; Borch, Kim; Thellersen, Marianne; Patkar, Shamkant Anant; Petersen, Dorte Aaby; Royer, John C.; Kretzschmar, Titus

PATENT ASSIGNEE(S): Novo Nordisk A/s, Den.; Okkels, Jens Sigurd; Svendsen, Allan; Borch, Kim; Thellersen, Marianne; Patkar, Shamkant Anant; Petersen, Dorte Aaby; Royer, John C.; Kretzschmar, Titus

SOURCE: PCT Int. Appl., 275 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9707202	A1	19970227	WO 1996-DK341	19960812
W:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA			
AU 9666551	A1	19970312	AU 1996-66551	19960812
EP 851913	A1	19980708	EP 1996-926323	19960812
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI			
CN 1192780	A	19980909	CN 1996-196233	19960812
JP 11510699	T2	19990921	JP 1996-508840	19960812
PRIORITY APPLN. INFO.:			DK 1995-905	19950811
			DK 1995-1096	19950929
			US 1996-11627	19960214
			DK 1996-374	19960401
			US 1996-16754	19960507
			WO 1996-DK341	19960812

AB Novel lipolytic enzymes are disclosed which are capable of removing substantial amts. of lard from a lard-stained swatch in a one-cycle wash performed under realistic washing conditions. Preferred lipolytic enzymes are variants of the *Humicola lanuginosa* lipase which may be prepd. by recombinant DNA techniques. Random mutagenized libraries of the entire *H. lanuginosa* lipase gene and of the lid domain (amino acids 91-97) and hydrophobic cleft region (amino acids 206-211), regions known to be important for wash performance, were constructed and screened using the Dobanol 25-7 and low-calcium assays. Twenty variants having very good washing performance were allowed to recombine by an in vivo recombination method in *Saccharomyces cerevisiae* YNG318. N-terminal peptides with low susceptibility to proteolytic degradn. were added to the lipase muteins and further subjected to random mutagenesis. Cloning and fermin. procedures

are described for the prodn. of the *H. lanuginosa* lipase muteins in *Aspergillus oryzae* and *Fusarium graminearum*. **Construction** of *Absidia reflexa* and *Pseudomonas* lipase mutants is also described. The enzymes are advantageously used in detergent compns. Thus, the *H. lanuginosa* lipase contg. an N-terminal SPIRPRP peptide replacing the E1 residues, and the substitutions D57G, N94K, D96L, L97M and Q249R removed 46% of lard at 12,500 Units/L, in comparison to 0-7% removal by various com. lipases.

IT 9001-92-7, Proteinase 9003-99-0, Peroxidase

RL: NUU (Nonbiological use, unclassified); USES (Uses)

(detergent additive; lipolytic enzyme muteins designed for one-wash detergent compns. for the removal of fatty materials)

L26 ANSWER 14 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:4458 HCAPLUS

DOCUMENT NUMBER: 126:70969

TITLE: Cloning of the polyketide **synthase** gene atX from *Aspergillus terreus* and its identification as the 6-methylsalicylic acid **synthase** gene by heterologous expression

AUTHOR(S): Fujii, I.; Ono, Y.; Tada, H.; Gomi, K.; Ebizuka, Y.; Sankawa, U.

CORPORATE SOURCE: Fac. Pharmaceutical Sciences, Univ. Tokyo, Tokyo, 113, Japan

SOURCE: Mol. Gen. Genet. (1996), 253(1-2), 1-10
CODEN: MGGEAE; ISSN: 0026-8925

PUBLISHER: Springer

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Southern blot anal. of genomic DNAs of several fungi that produce polyketide compds. with the 6-methylsalicylic acid **synthase** (MSAS) gene of *Penicillium patulum* as a probe indicated the presence of an MSAS-homologous gene in the (+)-geodin-producing strain IMI 16043 of *Aspergillus terreus*. The gene, designated atX was cloned from an *A. terreus* genomic **DNA library** and 7588 bp of the gene together with its flanking regions were sequenced to reveal the presence of a 5.5 kb open reading frame coding for a protein of 1800 amino acids with 190 kDa mol. mass. The presence of a short (70 bp) intron near the N-terminus of the atX gene was predicted that contains the canonical GT and AG **dinucleotides** at its 5'- and 3'-splicing junctions. The predicted ATX polypeptide showed high homol. with *P. patulum* MSAS along the whole sequence. On the other hand, slight homol. was detected only around the .beta.-ketoacyl **synthase** regions of *Aspergillus nidulans* wA, PKSST and *Colletotrichum lagenarium* PKS1. No transcription of atX was obsd. throughout the culture period by Northern blotting anal. To identify the function of the polypeptide encoded by the atX gene, its ordering region was introduced into the fungal expression **vector** pTAex3 under the control of the amyB promoter. The **constructed** explosion plasmid was introduced into *A. nidulans*. The transformant produced significant amts. of 6-methylsalicylic acid, the structure of which was identified by physicochem. anal. This result unambiguously demonstrated that the atX gene codes for MSAS of *A. terreus*.

IT 185261-08-9

RL: BAC (Biological activity or effector, except adverse); PRP (Properties); BIOL (Biological study)

(amino acid sequence; cloning of polyketide **synthase** gene atX from **Aspergillus terreus** and its identification as 6-methylsalicylic acid **synthase** gene by heterologous expression)

IT 185325-76-2

RL: PRP (Properties)

(amino acid sequence; cloning of polyketide **synthase** gene atX from **Aspergillus terreus** and its identification as 6-methylsalicylic acid **synthase** gene by heterologous expression)

IT 9045-37-8, 6-Methylsalicylic acid **synthase**

RL: BAC (Biological activity or effector, except adverse); PRP (Properties); BIOL (Biological study)

(gene atX; cloning of polyketide **synthase** gene atX from **Aspergillus terreus** and its identification as 6-methylsalicylic acid **synthase** gene by heterologous expression)

L26 ANSWER 15 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:180429 HCAPLUS

DOCUMENT NUMBER: 124:222465

TITLE: The *Gibberella fujikuroi* niaD gene encoding nitrate **reductase**: isolation, sequence, homologous transformation and electrophoretic karyotype location
AUTHOR(S): Tudzynski, Bettina; Mende, Katrin; Weltring, Klaus-Michael; Kinghorn, James R.; Unkles, Shiela E.
CORPORATE SOURCE: Institut fur Botanik und Botanischer Garten, Westfalische Wilhelms-Universitat, Munster, D-48149, Germany

SOURCE: Microbiology (Reading, U. K.) (1996), 142(3), 533-9
CODEN: MROBEO; ISSN: 1350-0872

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The *Gibberella fujikuroi* niaD gene, encoding nitrate **reductase**, has been isolated and used to develop an efficient homologous transformation system. A cosmid **vector** designated pGFniaD was generated based on niaD **selection** and shown to give comparable transformation efficiencies. Using pGFniaD, a genomic **library** was prep'd. and used for genetic transformations, giving frequencies of up to 200 transformants per .mu.g DNA. Of 15 transformants analyzed by Southern blots, six showed homologous integration while the remaining nine integrated at heterologous sites, indicating that the **vector** may be used reliably for both types of integration. The system therefore may be used both for self-cloning of gibberellin biosynthetic genes on the basis of complementation of defective mutants, and also for gene disruption expts. Electrophoretic karyotype detn. suggested at least 11 chromosomes ranging from 2 to 6 Mb, the total genome size being at least 37 Mb. The niaD gene was assigned to chromosome V by Southern blot anal. The niaD gene is interrupted by one intron, and remarkably the promoter sequence, but not the 3' untranslated sequence, is highly homologous to that of the corresponding *Fusarium oxysporum* gene. This situation appears to be unique with respect to the promoter regions of corresponding genes in related species of **filamentous fungi**.

IT 9013-03-0, Nitrate **reductase**

RL: PRP (Properties)

(*Gibberella fujikuroi* niaD gene encoding nitrate **reductase**:

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isolation, sequence, homologous transformation and electrophoretic karyotype location)

IT 174821-36-4

RL: PRP (Properties)

(amino acid sequence; Gibberella fujikuroi niaD gene encoding nitrate

reductase: isolation, sequence, homologous transformation and electrophoretic karyotype location)

L26 ANSWER 16 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:95136 HCAPLUS

DOCUMENT NUMBER: 124:137850

TITLE: Cloning and characterization of gene GLS1 encoding 1,3-.beta.-D-glucan **synthase** from Saccharomyces cerevisiae and its use in **screening** from antifungal compounds

INVENTOR(S): El-Sherbeini, Mohamed; Clemas, Joseph A.

PATENT ASSIGNEE(S): Merck and Co., Inc., USA

SOURCE: PCT Int. Appl., 55 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9532982	A1	19951207	WO 1995-US6557	19950522
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5484724	A	19960116	US 1994-249420	19940526
CA 2191067	AA	19951207	CA 1995-2191067	19950522
EP 763046	A1	19970319	EP 1995-922883	19950522
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
JP 10501408	T2	19980210	JP 1995-501009	19950522
US 5955337	A	19990921	US 1996-737663	19961121
PRIORITY APPLN. INFO.:			US 1994-249420	19940526
			WO 1995-US6557	19950522

AB The **DNA** encoding glucan synthesis gene 1 (GLS1) is cloned and used in an in vitro assay to **screen** for compds. that modulate 1,3-.beta.-D-glucan **synthase** activity. Thus, the potent echinocandin L-733,560 was used as a **selective** agent to isolate mutant strains of Saccharomyces cerevisiae specifically affected in glucan synthesis. One mutant (strain MS14) is echinocandin-resistant and is also supersensitive to the chitin **synthase** inhibitor nikkomycin Z. The mutation in MS14 maps to the FKS1 gene and is designated fks1-4. Another mutant (Strain MS1) is resistant to echinocandins and supersensitive to both papulacandin and rapamycin. Strain MS1 was used to clone the GLS1 gene encoding 1,3-.beta.-D-glucan **synthase** from a yeast genomic **DNA library**. GLS1 was cloned for expression of the GLS1 polypeptide in other host cell systems and in a process for prodn. of a glucan **synthase** subunit peptide. Hybridization analyses detected GLS1 homologs in other fungal species, such as Candida albicans, **Aspergillus fumigatus**, Schizosaccharomyces pombe, Phytophthora infestans, and Pneumocystis carinii. GLS1 and fks1-4 mutants can be incorporated into an assay to **screen** and classify antifungal compds. with chitin and glucan

synthase inhibitory effects, based on their differential resistance/sensitivity to the echinocandins, papulacandin, and nikkomycin Z.

- IT **133105-78-9P**, Protein (Saccharomyces cerevisiae clone E5F 347-amino acid reduced)
 RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses) (amino acid sequence; cloning and characterization of gene GLS1 encoding 1,3-.beta.-D-glucan **synthase** from Saccharomyces cerevisiae and its use in **screening** from antifungal compds.)
- IT **9037-30-3P**, 1,3-.beta.-D-Glucan **synthase**
 RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses) (cloning and characterization of gene GLS1 encoding 1,3-.beta.-D-glucan **synthase** from Saccharomyces cerevisiae and its use in **screening** from antifungal compds.)

L26 ANSWER 17 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:847293 HCAPLUS

DOCUMENT NUMBER: 124:46919

TITLE: Cloning and characterization of three **Aspergillus niger** promoters

AUTHOR(S): Luo, Xinmei

CORPORATE SOURCE: Max Planck Research Group SIWA, Friedrich-Schiller University Jena, D-07747, Jena, Germany

SOURCE: Gene (1995), 163(1), 127-31
 CODEN: GENED6; ISSN: 0378-1119

DOCUMENT TYPE: Journal

LANGUAGE: English

AB An **Aspergillus niger** (An) genomic library was constructed using the promoter-trap vector, pLX2A, which contains a hygromycin B (Hy) **phosphotransferase**-encoding gene (hph) for selection of DNA fragments with promoter activity. This library was transformed in Escherichia coli and 80000 colonies were obtained, 94% of which contained inserts. Transformations of plasmid DNA from the library into An resulted in 53 Hy-resistant (HyR) colonies. Southern blot anal. of 21 transformants confirmed the integration of hph into the An genome. Using the sib selection procedure, three functional promoters, PX6, PX18 and PX21, were identified from this library. Both DNA strands of all three fragments were sequenced and their sequences showed no significant homol. to those in the database. Comparison of the sequences of all known promoters from An suggested that C+T-rich stretches are probably important for promoter structures. The promoter activity was analyzed further using .beta.-galactosidase (.beta.Gal) as a quant. marker. The results suggest that while PX21 is a much stronger promoter than the known .alpha.-amylase promoter of A. oryzae, PX6 promotes only weak expression of .beta.Gal.

IT **141008-60-8**, GenBank M90701 **141008-62-0**, GenBank M90699

141008-63-1, GenBank M90700

RL: BOC (Biological occurrence); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)

(nucleotide sequence; cloning and characterization of three **Aspergillus niger** promoters)

L26 ANSWER 18 OF 30 HCAPLUS COPYRIGHT 2001 ACS

M. Smith 308-3278

ACCESSION NUMBER: 1995:286688 HCAPLUS
DOCUMENT NUMBER: 122:98005
TITLE: A cosmid with a HyR **marker** for fungal
library construction and
screening

AUTHOR(S): Orbach, Marc J.
CORPORATE SOURCE: Dupont Experimental Station, Wilmington, DE,
19880-0402, USA
SOURCE: Gene (1994), 150(1), 159-62
CODEN: GENED6; ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **construction** of a double-cos-site cosmid **vector**, pMOcosX, for use in making **filamentous fungal** genomic **DNA libraries**, is described. The **vector** has features that allow for **selection** of clones introduced into fungi by transformation and for efficient chromosome walking expts. These features (i) two cos sites allowing for easy **construction** of **libraries** without requiring size **selection** of insert **DNA**; (ii) an XhoI site for insertion of Sau3AI or MboI partially digested genomic **DNA** inserts that allows usage of a half-site fill-in method which minimizes the possibility of producing clones contg. chimeric inserts; (iii) a bacterial hygromycin **phosphotransferase** -encoding gene fused to a modified cpc-1 promoter of Neurospora crassa for direct **selection** of cosmid clones upon introduction into fungal cells; and (iv) T7 and T3 bacteriophage promoters and EcoRI, NotI and BamHI restriction sites flanking the cloning site that allow for synthesis of, or isolation of, end-specific probes for chromosome walking. The combination of features in this **vector** allows for the easy **construction** and use of high-quality fungal **DNA libraries** from small amts. of genomic **DNA**.

L26 ANSWER 19 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:500829 HCAPLUS
DOCUMENT NUMBER: 121:100829
TITLE: A versatile shuttle cosmid **vector** for the
efficient **construction** of genomic
libraries and for the cloning of fungal genes

AUTHOR(S): Osiewacz, Heinz D.
CORPORATE SOURCE: Angewandte Tumorstudiologie, Deutsches
Krebsforschungszentrum, Heidelberg, D-69120, Germany
SOURCE: Curr. Genet. (1994), 26(1), 87-90
CODEN: CUGED5; ISSN: 0172-8083
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A shuttle cosmid **vector**, pANsCos1, has been **constructed** for Escherichia coli and **filamentous fungi**. This **vector** contains two cos sequences sepd. by a single XbaI restriction site. pANsCos1 allows the efficient **construction** of representative genomic **libraries** from as little as 15-20 .mu.g of genomic **DNA**. Due to the presence of a functional hygromycin B **phosphotransferase** gene (hph), transformation of fungal protoplasts with pANsCos1, or derivs. of it, results in the formation of hygromycin B-resistant transformants. The T7 and T3 **RNA** polymerase promoter sequences flanking the cloning site, in combination with two adjacent NotI sites facilitate genomic walking and the rapid

IT 88361-67-5, Hygromycin B **phosphotransferase**
 RL: BIOL (Biological study)
 (gene hph for, on cosmid shuttle **vector** for cloning of fungal
 genes in Escherichia coli and **filamentous fungi**)

L26 ANSWER 20 OF 30 HCAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 1994:428090 HCAPLUS
 DOCUMENT NUMBER: 121:28090
 TITLE: An "instant gene bank" method for heterologous gene
 cloning: complementation of two **Aspergillus**
 nidulans mutants with Gaeumannomyces graminis

AUTHOR(S): **DNA** Bowyer, P.; Osbourn, A. E.; Daniels, M. J.
 CORPORATE SOURCE: Sainsbury Lab., Colney/Norwich, NR47UH, UK
 SOURCE: Mol. Gen. Genet. (1994), 242(4), 448-454
 CODEN: MGGEAE; ISSN: 0026-8925

DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The authors present a novel technique for gene cloning by complementation
 of mutations in **Aspergillus** nidulans with **DNA** from a
 heterologous organism, Gaeumannomyces graminis. This technique bypasses
 the time-consuming and difficult **construction** of gene
libraries, making it both rapid and simple. The method relies on
 recombination between a fungal replicating **vector** pHELP1 and
 linear G. graminis genomic **DNA** during co-transformation. The
 authors were able to complement two out of seven A. nidulans mutants
 tested and to rescue transforming **DNA** from both in Escherichia
 coli. Complementation of the A. nidulans argB mutation resulted from
 integration of 8-10 kb segments of G. graminis **DNA** into pHELP1.
 The complementation of the A. nidulans pyrG mutation resulted from a
 complex rearrangement. Complementing **DNA** was shown to originate
 from G. graminis, and was capable of retransforming the original mutants
 to give the expected phenotype.

IT 9001-69-8, Ornithine **carbamoyltransferase**
 RL: BIOL (Biological study)
 (gene argB for, of Gaeumannomyces graminis, instant gene bank method
 for cloning of, in **Aspergillus** nidulans)

L26 ANSWER 21 OF 30 HCAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 1994:293008 HCAPLUS
 DOCUMENT NUMBER: 120:293008
 TITLE: A subtilisin-like serine proteinase from
Aspergillus and the gene encoding it

INVENTOR(S): Buxton, Frank
 PATENT ASSIGNEE(S): Ciba-Geigy A.-G., Switz.
 SOURCE: Can. Pat. Appl., 83 pp.
 CODEN: CPXXEB

DOCUMENT TYPE: Patent
 LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2093950	AA	19931016	CA 1993-2093950	19930413

M. Smith 308-3278

EP 574347 A2 19931215 EP 1993-810243 19930406
 EP 574347 A3 19940413
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE
 NO 9301368 A 19931018 NO 1993-1368 19930414
 ZA 9302611 A 19931026 ZA 1993-2611 19930414
 JP 06046863 A2 19940222 JP 1993-86632 19930414
 HU 67800 A2 19950529 HU 1993-1087 19930414
 AU 9336959 A1 19931021 AU 1993-36959 19930415
 AU 663173 B2 19950928
 PRIORITY APPLN. INFO.: EP 1992-810281 19920415
 GB 1993-5097 19930312

AB The DNA sequence coding for an *Aspergillus* subtilisin-like serine protease and the enzyme and its prepn. are described. A novel *Aspergillus* mutant defective in such a serine protease and therefore useful for the manuf. of heterologous proteins, and a method for its prepn. are described. An *Aspergillus niger* NdeI partial digest library in .lambda.EMBL4 was screened with the yeast PRB gene and the cloned sequence transferred to the com. vector pTZ18R for further manipulation. The endogenous proteinase gene (pepC) of *A. niger* was disrupted by homologous recombination with introduction of the pyrA gene. Using PCR primers derived from conserved sequences, a second gene (pepD) encoding a second such proteinase was obtained.

IT 148155-53-7 154768-22-6
 RL: BIOL (Biological study)
 (amino acid sequence of and cloning and expression of gene for)
 IT 9001-59-6, Pyruvate kinase
 RL: BIOL (Biological study)
 (gene for, promoter of, of *Aspergillus niger*, expression of pepC gene of *A. niger* using)
 IT 147904-13-0, Deoxyribonucleic acid (*Aspergillus niger* clone pANB1 gene pepC plus 5'- and 3'-flanking region fragment)
 154688-94-5
 RL: BIOL (Biological study)
 (nucleotide sequence of and cloning and expression and disruption of)

L26 ANSWER 22 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:186873 HCAPLUS

DOCUMENT NUMBER: 120:186873

TITLE: Polyhydroxynaphthalene reductase involved in melanin biosynthesis in *Magnaporthe grisea*.

Purification, cDNA cloning and sequencing
 AUTHOR(S): Vidal-Cros, Anne; Viviani, Fabrice; Labesse, Gilles; Boccara, Martine; Gaudry, Michel

CORPORATE SOURCE: Lab. Chim. Org. Biol., CNRS, Paris, Fr..

SOURCE: Eur. J. Biochem. (1994), 219(3), 985-92

CODEN: EJBCAI; ISSN: 0014-2956

DOCUMENT TYPE: Journal

LANGUAGE: English

AB During the biosynthesis of fungal melanin, tetrahydroxynaphthalene reductase catalyzes the NADPH-dependent redn. of 1,3,6,8-tetrahydroxynaphthalene (T4HN) into (+)-scytalone and 1,3,8-trihydroxynaphthalene into (-)-vermelone. The enzyme from *Magnaporthe grisea*, the fungus responsible for rice blast disease, has been purified to homogeneity. It is a tetramer of four identical 30-kDa

subunits. A full-length cDNA clone of about 1 kb encoding T4HN **reductase** has been isolated from a cDNA library **constructed** in the .lambda.ZAP II **vector** and characterized. The clone contains a 846-bp open reading frame. Translation of the **DNA** sequence gave a 282-residue amino acid sequence with a calcd. mol. mass of 29.9 kDa. Sequences corresponding to the amino-terminal part and three internal proteolytic peptides were present in the translated sequence. T4HN **reductase** exhibits characteristics of the short-chain alc. dehydrogenase family. The **reductase** shares 56% identity with a putative ketoreductase involved in aflatoxin biosynthesis in **Aspergillus parasiticus**.

IT 152002-17-0

RL: PRP (Properties)

(nucleotide sequence of)

IT 153702-05-7P, Tetrahydroxynaphthalene **reductase**

(Magnaporthe grisea clone pAV501) (E.C.1.3.1.50)

RL: PRP (Properties); PREP (Preparation)

(purifn. and amino acid sequence of)

L26 ANSWER 23 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:155921 HCAPLUS

DOCUMENT NUMBER: 120:155921

TITLE: The ornithine carbamoyl **transferase** gene of the white rot fungus *Coriolus hirsutus* and its utilization

INVENTOR(S): Tsukamoto, Akira; Matsufuji, Mieko; Kita, Yukio

PATENT ASSIGNEE(S): Oji Paper Co., Ltd., Japan

SOURCE: Can. Pat. Appl., 54 pp.

CODEN: CPXXEB

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2091236	AA	19930910	CA 1993-2091236	19930308
EP 570096	A2	19931118	EP 1993-301737	19930308
EP 570096	A3	19960103		
R: DE, FR, GB, IT				
JP 06054691	A2	19940301	JP 1993-46798	19930308
US 5362640	A	19941108	US 1993-27986	19930308
PRIORITY APPLN. INFO.:			JP 1992-50513	19920309
			JP 1992-104549	19920423

AB The gene for the ornithine carbamoyl **transferase** (OCT) of *Coriolus hirsutus* is cloned and characterized for use in the development of expression **vectors** and hosts for basidiomycetes. Methods for use of arginine auxotrophs as hosts and for successful transformation of *C. hirsutus* are described. The method is particularly useful in the manuf. of lignin peroxidase. A cDNA for the OCT gene was cloned by **screening** a library with probes derived from the *argB* gene of *Aspergillus nidulans* and the cDNA used to **screen** a Sau3A partial digest genomic bank in .lambda.EMBL3. Arg- and Arg-Leu-auxotrophs were prepd. for use as transformation hosts by mutagenesis and **screening**. Transformation was achieved using mycelial or oidial protoplasts and polyethylene glycol with a transformation frequency of 300

colonies/.mu.g DNA. Successful transformation with the lignin peroxidase gene of *C. hirsutus* with the peroxidase gene under control of its own promoter or the OCT promoter was demonstrated with the enzyme accumulating in the medium at 20-100 units/mL. The signal sequences of the *C. hirsutus* phenol oxidase gene was used to direct secretion.

Construction of expression **vectors** for phenol oxidase and luciferase genes was also demonstrated.

IT 153571-48-3 153571-49-4

RL: BIOL (Biological study)

(amino acid sequence of and cloning and expression of gene for, transformation **marker** in relation to)

IT 9001-69-8, Ornithine carbamoyl **transferase**

RL: BIOL (Biological study)

(gene for, of *Coriolus hirsutus*, cloning of, in development of *C. hirsutus* as basidiomycete expression host)

IT 9002-10-2, Phenol oxidase

RL: BIOL (Biological study)

(gene for, of *Coriolus hirsutus*, expression in *C. hirsutus* of, transformation in relation to)

IT 153571-50-7

RL: BIOL (Biological study)

(**nucleotide** sequence and cloning and expression of, as **selectable marker** in transformation)

IT 153571-51-8

RL: PRP (Properties); BIOL (Biological study)

(**nucleotide** sequence and cloning of)

L26 ANSWER 24 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1993:669188 HCAPLUS

DOCUMENT NUMBER: 119:269188

TITLE: Manufacture of **Aspergillus niger** catalase R
by expression of the gene from the glucoamylase
promoter

INVENTOR(S): Berka, Randy M.; Fowler, Timothy; Rey, Michael W.

PATENT ASSIGNEE(S): Genencor International, Inc., USA

SOURCE: PCT Int. Appl., 42 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9318166	A2	19930916	WO 1993-US2020	19930304
WO 9318166	A3	19931028		
W: CA, FI, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5360732	A	19941101	US 1992-846181	19920304
US 5360901	A	19941101	US 1992-845989	19920304
EP 630408	A1	19941228	EP 1993-907261	19930304
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 07504332	T2	19950518	JP 1993-515925	19930304
FI 9404051	A	19940902	FI 1994-4051	19940902
PRIORITY APPLN. INFO.:			US 1992-845989	19920304
			US 1992-846181	19920304

WO 1993-US2020 19930304

AB The catalase R product of the catR gene of **Aspergillus niger** is manufd. without the need to use hydrogen peroxide induction by placing the gene under control of the glucoamylase (glaA) gene promoter and deletion of the glucose oxidase (goxA) gene. This method also minimizes yields of sodium gluconate. A cDNA for the catalase was cloned by **screening** a .lambda.gt11 **library** with amino acid sequence-derived **oligonucleotides** and used to **screen** a genomic **library**. The gene was placed under control of the glaA promoter and placed alongside the pyrG gene (**selectable marker**) and introduced into a .DELTA.goxA pyrG metC host. Two transformants showed catalase levels 10-15-fold higher than the wild-type. Sodium gluconate levels were 48-123 vs. >200,000 mg/L for the parental strain.

IT 151500-58-2

RL: BIOL (Biological study)
(amino acid sequence and cloning and expression in **Aspergillus niger** of gene for)

IT 9032-08-0, Glucoamylase

RL: BIOL (Biological study)
(gene for, promoter of, of **Aspergillus niger**, expression of catR catalase gene from)

IT 151500-57-1

RL: BIOL (Biological study)
(**nucleotide** sequence and cloning and expression in **Aspergillus niger** of)

IT 9001-37-0, Glucose oxidase

RL: BIOL (Biological study)
(goxA gene for, deletion of, in **Aspergillus niger** hosts for high-level expression of catR catalase gene)

L26 ANSWER 25 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1993:206328 HCAPLUS

DOCUMENT NUMBER: 118:206328

TITLE: Sequence analysis of the gene coding for glyceraldehyde-3-phosphate dehydrogenase (gpd) of *Podospora anserina*: use of homologous regulatory sequences to improve transformation efficiency

AUTHOR(S): Ridder, Ruediger; Osiewacz, Heinz D.

CORPORATE SOURCE: Abt. Molekularbiol. Alterungsprozesse, Dtsch. Krebsforschungszent., Heidelberg, W-6900, Germany

SOURCE: Curr. Genet. (1992), 21(3), 207-13

CODEN: CUGED5; ISSN: 0172-8083

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The glyceraldehyde-3-phosphate dehydrogenase (gpd) gene of *P. anserina* was isolated from a genomic **library** by heterologous hybridization with the corresponding gene of *Curvularia lunata*. The coding region consists of 1-14 **nucleotides** and is interrupted by a single intron. The amino-acid sequence encoded by the gpd gene shows a high degree of sequence identity with the corresponding gene products of various fungi. Multiple alignments of all fungal GPD sequences so far available resulted in the **construction** of a phylogenetic tree. The evolutionary relationships of the various fungi belonging to different taxa will be discussed on the basis of these data. Sequence anal. of 1.9 kbp of the 5' non-coding region revealed the presence of typical fungal promoter elements. Utilizing different parts of the 5' regulatory

sequence of the *Podospora* *gpd* gene, expression **vectors** contg. a dominant **selectable marker** gene (hygromycin B **phosphotransferase**) were **constructed** for the transformation of *P. anserina* protoplasts. The use of these homologous *gpd* regulatory sequences resulted in a significant increase in transformation efficiencies compared to those obtained with **vectors** in which the **selectable marker** gene is under the control of the corresponding heterologous promoter of *Aspergillus nidulans*.

L26 ANSWER 26 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1991:672470 HCAPLUS

DOCUMENT NUMBER: 115:272470

TITLE: Cloning of the nitrate - nitrite **reductase** gene cluster of *Penicillium chrysogenum* and use of the *niaD* gene as a homologous **selection marker**

AUTHOR(S): Gouka, Robin J.; Van Hartingsveldt, Wim; Bovenberg, Roel A. L.; Van den Hondel, Gees A. M. J. J.; Van Gorcom, Robert F. M.

CORPORATE SOURCE: Med. Biol. Lab., TNO, Rijswijk, NL-2280 AA, Neth.

SOURCE: J. Biotechnol. (1991), 20(2), 189-99

CODEN: JBTD4; ISSN: 0168-1656

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A new homologous transformation system for the **filamentous fungus** *P. chrysogenum* is described. The system is based on complementation of *niaD* mutants using the nitrate **reductase** structural gene (*niaD*) of *P. chrysogenum*. Spontaneous *niaD* mutants were identified after **selection** for chlorate resistance, in growth tests and subsequent complementation with the *niaD* gene of *Aspergillus oryzae*. The *P. chrysogenum* *niaD* gene was isolated from a genomic **library** using the *A. nidulans* *niaD* gene as a probe. After subcloning of the hybridizing fragment, the **vector** obtained, pPC1-1, was capable of transforming a *P. chrysogenum* *niaD* mutant at an av. of 40 transformants per .mu.g of circular **DNA**. Southern anal. of genomic **DNA** from a no. of transformants showed that pPC1-1 **DNA** was integrated predominantly at sites other than the *niaD* locus. Using hybridization anal. it was shown that the *niaD* gene of *P. chrysogenum* is clustered with the nitrite **reductase** gene (*niiA*). From anal. of the **nucleotide** sequences of parts of the *niaD* and *niiA* genes of *P. chrysogenum* and comparison of these sequences with **nucleotide** sequences of the corresponding *A. nidulans* gene it was deduced that the *P. chrysogenum* genes are divergently transcribed.

IT 9013-03-0, Nitrate **reductase**

RL: PRP (Properties)

(gene for, of *Penicillium chrysogenum*, cloning and sequence of and homologous transformation system based on complementation of mutations in)

IT 9080-03-9, Nitrite **reductase**

RL: PRP (Properties)

(gene for, of *Penicillium chrysogenum*, cloning of and divergent transcription of nitrate **reductase** gene and)

L26 ANSWER 27 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1991:529050 HCAPLUS

DOCUMENT NUMBER: 115:129050
 TITLE: Genetic transformation of auxotrophic mutants of the filamentous yeast *Trichosporon cutaneum* using homologous and heterologous **marker** genes
 AUTHOR(S): Ochsner, Urs A.; Glumoff, Virpi; Kaelin, Markus; Fiechter, Armin; Reiser, Jakob
 CORPORATE SOURCE: Inst. Biotechnol., ETH-Hoenggerberg, Zurich, CH-8093, Switz.
 SOURCE: Yeast (1991), 7(5), 513-24
 CODEN: YESTE3; ISSN: 0749-503X
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB A transformation system for the filamentous yeast *T. cutaneum* based on auxotrophic **markers** is presented and techniques for the induction, isolation and characterization of mutants are described. A no. of auxotrophic mutants were isolated and characterized by using biosynthetic precursors and/or inhibitors. A mutant unable to grow in the presence of ornithine could be complemented successfully in spheroplast transformation expts. using the cloned *Aspergillus nidulans* ornithine transcarbamoylase gene (*argB* gene) as **selection marker** with an efficiency of 5-100 transformants per .mu.g of **DNA**. In these transformants the heterologous *argB* gene was present in multiple tandem copies and the transforming **DNA** was found to remain stable after more than 50 generations in non-selective media. The same mutant could be complemented by a *T. cutaneum* cosmid gene **library** and a complementing cosmid was subsequently isolated from this **library** by a sib-selection strategy. This cosmid transformed *T. cutaneum* spheroplasts with an efficiency of 500-200 colonies per .mu.g of **DNA**. Southern blot anal. were consistent with the view that the transforming sequences became stably integrated into the host genome at the homologous site.

IT 9001-69-8, Ornithine carbamoyl **transferase**
 RL: PRP (Properties)
 (Trichosporon cutaneum mutants deficient in, isolation and transformation of)

IT 9023-58-9, Argininosuccinate synthetase 9026-23-7, Carbamoyl phosphate synthetase
 RL: PRP (Properties)
 (Trichosporon cutaneum mutants deficient in, isolation of)

L26 ANSWER 28 OF 30 HCAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 1990:566790 HCAPLUS
 DOCUMENT NUMBER: 113:166790
 TITLE: Homologous transformation of *Cephalosporium acremonium* with the nitrate **reductase**-encoding gene (*niaD*)
 AUTHOR(S): Whitehead, Michael P.; Gurr, Sarah Jane; Grieve, Carolyn; Unkles, Shiela E.; Spence, David; Ramsden, Martin; Kinghorn, James R.
 CORPORATE SOURCE: Plant Mol. Genet. Unit, Univ. St. Andrews, Fife, KY16 9TH, UK
 SOURCE: Gene (1990), 90(2), 193-8
 CODEN: GENED6; ISSN: 0378-1119
 DOCUMENT TYPE: Journal
 LANGUAGE: English

- AB The development is reported of a homologous transformation system for *C. acremonium* using the *niaD* gene of the nitrate assimilation (NA) pathway. Mutants in the NA pathway were **selected** on the basis of chlorate resistance by conventional means. **Screening** procedures were developed to differentiate between nitrate **reductase** apoprotein structural gene mutants (*niaD*) and molybdenum cofactor gene mutants (*cnx*) as wild-type *C. acremonium*, unlike most **filamentous fungi**, fails to grow on minimal medium with hypoxanthine as a sole source of nitrogen. Phage clones carrying the *niaD* gene were isolated from a *C. acremonium* **library constructed** in λ .EMBL3 using the *A. nidulans* *niaD* gene as a heterologous probe. An 8.6-kb EcoRI fragment was subcloned into pUC18, and designated pSTA700. pSTA700 was able to transform stable *niaD* mutants to NA at a frequency of up to 40 transformants per μ g DNA. Transformants were easily visible since the background growth was low and no abortives were obsd. Gene replacements, single copy homologous integration, and complex multiple integrations were obsd. The *niaD* system was used to introduce unselected **markers** for hygromycin B resistance and benomyl resistance into *C. acremonium* by cotransformation.
- IT **9023-03-4, Cytochrome c reductase**
 RL: PRP (Properties)
 (activity of, of *Cephalosporium acremonium* transformants, **screening** procedure for transformation with nitrate **reductase** gene in relation to)
- IT **9013-03-0, Nitrate reductase**
 RL: PRP (Properties)
 (gene *niaD* for, of *Cephalosporium acremonium*, homologous transformation with, cotransformation in relation to)

L26 ANSWER 29 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1990:230812 HCAPLUS

DOCUMENT NUMBER: 112:230812

TITLE: Cloning of a new bidirectionally **selectable marker** for ***Aspergillus*** strains

AUTHOR(S): Buxton, Frank P.; Gwynne, David I.; Davies, R. Wayne

CORPORATE SOURCE: Allelix Biopharm., Mississauga, ON, L4V 1P1, Can.

SOURCE: Gene (1989), 84(2), 329-34

CODEN: GENED6; ISSN: 0378-1119

DOCUMENT TYPE: Journal

LANGUAGE: English

- AB Mutants that lack ATP sulfurylase (ATPase; EC 2.7.7.4) are unable to use sulfate as sole source of sulfur and are also resistant to selenate. These mutants, denoted sC-, are readily obtained from any strain of *A. niger* or *A. nidulans* by the strong **selection** for selenate resistance. The gene encoding ATPase was cloned from *A. nidulans* by complementation of an sC mutant strain of *A. nidulans* with a gene **library**. Plasmids contg. this gene transform both *A. niger* and *A. nidulans* sC- strains, restoring their ability to grow on sulfate as sole sulfur source. The fact that strong **selection** for either sC+ or sC- can be applied provides a simple way of delivering genetically engineered **constructs** to any strain of *A. niger* including strains of industrial importance. In addn., this system is useful for gene replacements and other genomic DNA manipulations in ***Aspergillus*** species.
- IT **9012-39-9, ATP sulfurylase**
 RL: PRP (Properties)

(gene for, of *Aspergillus nidulans*, cloning and
selectable marker use of)

L26 ANSWER 30 OF 30 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1989:588736 HCAPLUS

DOCUMENT NUMBER: 111:188736

TITLE: The 5'-sequence of the isopenicillin N-synthetase gene
(pcbC) from *Cephalosporium acremonium* directs the
expression of the prokaryotic hygromycin B
phosphotransferase gene (hph) in
Aspergillus niger

AUTHOR(S): Kueck, Ulrich; Walz, Markus; Mohr, Georg; Mracek,
Miroslav

CORPORATE SOURCE: Ruhr-Univ. Bochum, Bochum, 4630/1, Fed. Rep. Ger.

SOURCE: Appl. Microbiol. Biotechnol. (1989), 31(4), 358-65
CODEN: AMBIDG; ISSN: 0175-7598

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A comparative transcript anal. was performed with **RNA** from 2
different strains of *C. acremonium*, using synthetic
oligonucleotides as specific probes for the isopenicillin
N-synthetase gene (pcbC). Strain DSM 2353 shows a considerably higher
amt. of the pcbC transcript than strain ATCC 14553. Subsequently, a
genomic library from *C. acremonium* strain DSM 2353 **DNA**
was **constructed** using lambda **vector** EMBL4. Five
recombinant clones contg. identical copies of the pcbC gene as confirmed
by partial **DNA** sequencing were isolated. The 5' region of the
pcbC gene was fused with the prokaryotic gene for hygromycin B
phosphotransferase (hph) using a synthetic **oligonucleotide**
linker. The resulting plasmid pMW1 can be used for high-frequency
transformations of the **filamentous fungus**
Aspergillus niger (.apprxeq.10,000 transformants/.mu.g plasmid
DNA). From Southern hybridization anal. it can be concluded that
all transformants tested contain **vector DNA** integrated
into the genomic **DNA**. The expression of the prokaryotic hph
gene in *A. niger* was conclusively demonstrated with an assay specific for
hygromycin B **phosphotransferase**.

IT 88361-67-5, Hygromycin B **phosphotransferase**

RL: PRP (Properties)

(gene hgh for, fusion with *Cephalosporium acremonium* gene pcbC of,
Aspergillus niger transformation by)

IT 78642-31-6, Isopenicillin N synthetase

RL: PRP (Properties)

(gene pcbC for, of *Cephalosporium acremonium*, hygromycin
phosphotransferase gene fusion with, ***Aspergillus***
niger transformation in relation to)